Agricultural and Biological Chemistry

ISSN: 0002-1369 (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tbbb19

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To cite this article: Teiichiro Ito & Tadao Ishii (1966) Semisynthetic Penicillin, Agricultural and Biological Chemistry, 30:10, 1047-1053, DOI: 10.1080/00021369.1966.10858712

To link to this article: https://doi.org/10.1080/00021369.1966.10858712

Published online: 09 Sep 2014.

[Agr. Biol. Chem., Vol. 30, No. 10, p. 1047~1053, 1966]

Semisynthetic Penicillin

Part II.* Preparation and Properties of α-Alkylthio-cinnamyl Penicillins**, ***

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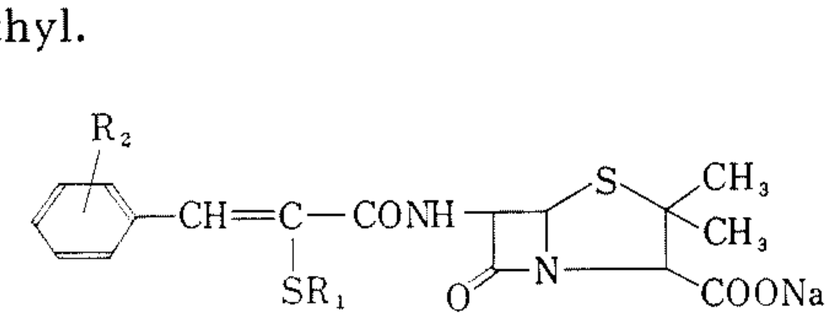
Received April 27, 1966

The structure-activity in α-alkylthio-cinnamyl penicillins was studied. These penicillins were prepared by condensing 6-aminopenicillanic acid with α-alkylthio-cinnamic acids. α-Methylthio-cinnamyl penicillin and its substituted analogues were highly inhibitory to *Staphylococcus aureus* 209P and some of them were also effective in vitro against benzyl-penicillin-resistant *Staphylococci*. *trans*-α-Methylthio-2-bromo-cinnamyl penicillin, which showed a good in vitro activity, was resistant to penicillinase and was stable in acidic aqueous solution.

Recently, numerous penicillinase-resistant penicillins have been prepared by acylating 6-aminopenicillanic acid (6-APA) with the chlorides of sterically hindered carboxylic acids. 2,6-Dimethoxyphenyl penicillin (methicillin)[1], 5-methyl-3-phenyl-4-isoxazolyl penicillin (oxacillin)[2] and its chlorinated derivatives (cloxacillin[3] and dicloxacillin[4]) and 2-ethoxy-1-naphtyl penicillin (nafcillin)[5] are now widely used in the treatment of benzylpenicillin-resistant staphylococcal infection.

In the previous communication,[6] we described the preparation of *trans*†-α-methylthio-2-chloro-cinnamyl penicillin (thiocillin) and *cis*†-α-methylthio-2-chloro-cinmamyl penicillin, which were stable to penicillinase. Thiocillin was effective in vitro against benzylpenicillin-sensitive microorganisms and also active against benzyl-penicillin-resistant *Staphylococci*. Microbiological studies and animal protection tests of this penicillin†† were described by Fukaya and Tomori.[7]

The present paper reports the structure-activity studies in α-alkylthio-cinnamyl penicillins. The general structure of these penicillins is represented by I, in which R_1 is methyl, ethyl, *n*-propyl and *n*-butyl and R_2 is hydrogen, chlorine, bromine, methoxy and methyl.



(I)

Preparative Method. These penicillins were synthesized by condensing α-alkylthio-cinnamic acids with 6-aminopenicillanic acid. The pre-

* Part I: *Chem. Pharm. Bull.*, **13**, 1148 (1965).

** This report was presented at the 21st Annual Meeting of Pharmaceutical Society of Japan, Tokushima, October 29, 1965.

*** Paper 5 in the series, Studies on Semisynthetic Penicillins. Paper 4. This Journal, **29**, 1119 (1965).

1) F.P. Doyle, K. Hardy, J.H.C. Nayler, M.J. Soulal, E.R. Stove and H.R.J. Waddington, *J. Chem. Soc.*, **1962**, 1453.

2) F.P. Doyle, A.A.W. Long, J.H.C. Nayler and E.R. Stove, *Nature*, **192**, 1183 (1961).

3) F.P. Doyle, J.C. Hanson, A.A.W. Long, J.H.C. Nayler and E.R. Stove, *J. Chem. Soc.*, **1963**, 5838.

4) P. Naumann and B. Kempf, *Arzneimittel-Forschung*, **15**, 139 (1965).

5) S.B. Rosenman and G.H. Warren, "Antimicrobial Agents and Chemotherapy-1961", p. 611.

6) T. Ito, T. Ishii and M. Nishio, *Chem. Pharm. Bull.*, **13**, 1148 (1965).

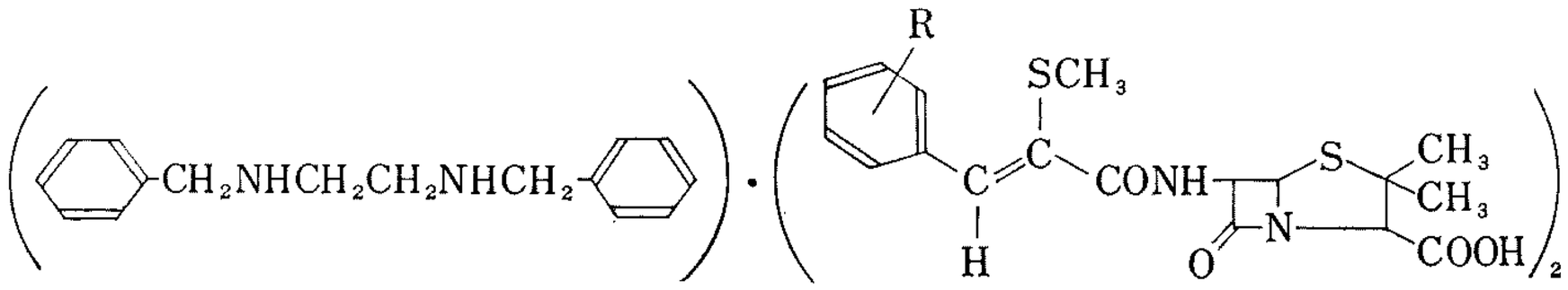
† The prefixes *trans* and *cis* refer to relationship between the carboxylic function and β-phenyl group of side chain.

†† The former name of thiocillin was P-18.

7) K. Fukaya and G. Tomori, *J. Antibiotics*, **B**, XVIII, 517 (1965).

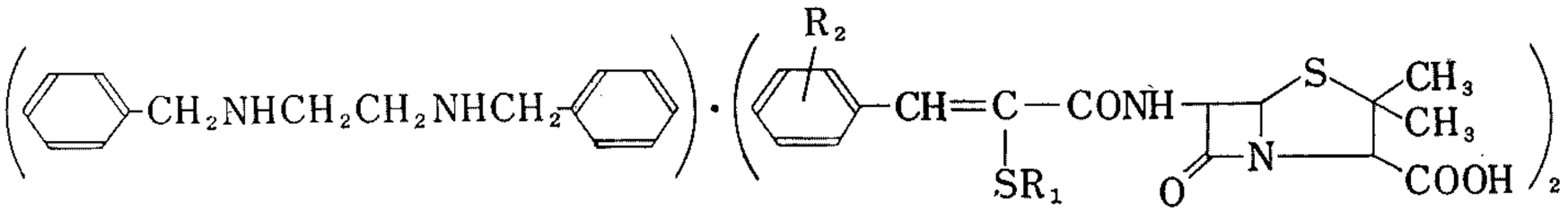
paration and geometrical isomerism of substituted α-methylthio-cinnamic acids (II) were reported previously.[8~10] α-Ethylthio-cinnamic acid (III), α-*n*-propylthio-cinnamic (IV) and α-*n*-butylthio-cinnamic acid (V) and their substituted derivatives were synthesized by alkylating β-aryl-α-thiopyruvic acids[8,10] with ethyl iodide,[11] *n*-propyl iodide and *n*-butyl iodide respectively. The properties of newly prepared acids are described in the Experimental section. The geometrical configuration of acids III, IV and V were not yet examined.

TABLE I. DIBENZYLETHYLENEDIAMINE SALTS OF *trans*-α-METHYLTHIO-CINNAMYL PENICILLINS



No.	R	Dec. temp. °C	Formula	*Anal.* Calcd. C	H	N	Found C	H	N
1	H	85~87	$C_{52}H_{60}O_8N_6S_4 \cdot H_2O$	59.86	5.99	7.85	59.98	6.15	7.38
2	2-Cl	130~150	Reference 6)						
3	3-Cl	74~90	$C_{52}H_{58}O_8N_6S_4Cl_2 \cdot 2H_2O$	55.26	5.53	7.43	55.62	6.04	7.70
4	4-Cl	77~90	$C_{52}H_{58}O_8N_6S_4Cl_2 \cdot 2H_2O$	55.26	5.53	7.43	55.71	6.32	7.36
5	3, 4-di-Cl	90	$C_{52}H_{56}O_8N_6S_4Cl_4 \cdot H_2O$	52.88	4.95	7.12	52.50	5.48	7.13
6	2, 6-di-Cl	96~105	$C_{52}H_{56}O_8N_6S_4Cl_4 \cdot H_2O$	52.88	4.95	7.12	52.39	5.30	7.09
7	2-Br	72~78	$C_{52}H_{58}O_8N_6S_4Br_2 \cdot 5H_2O$	49.05	5.38	6.60	48.88	5.29	7.09
8	4-Br	82~90	$C_{52}H_{58}O_8N_6S_4Br_2 \cdot 3H_2O$	50.48	5.21	6.79	50.77	5.45	6.31
9	2-OCH_3	78~85	$C_{54}H_{64}O_{10}N_6S_4$	58.67	5.83	7.60	58.27	5.96	7.54
10	2-CH_3	123~133	$C_{54}H_{64}O_8N_6S_4 \cdot 2H_2O$	58.46	6.18	7.57	58.00	6.52	7.45
11	4-CH_3	84~88	$C_{54}H_{64}O_8N_6S_4$	60.42	6.00	7.83	61.02	6.25	7.45

TABLE II. DIBENZYLETHYLENEDIAMINE SALTS OF α-ETHYLTHIO-, α-*n*-PROPYLTHIO- AND α-*n*-BUTYLTHIO-CINNAMYL PENICILLINS



No.	R_1	R_2	Dec. temp. °C	Formula	*Anal.* Calcd. C	H	N	Found C	H	N
12	C_2H_5	H	110~112	$C_{54}H_{64}O_8N_6S_4 \cdot 3H_2O$	58.57	6.37	7.58	58.95	6.29	7.08
13	*n*-C_3H_7	H	93~97	$C_{56}H_{68}O_8N_6S_4 \cdot 2H_2O$	60.19	6.18	7.52	60.42	6.20	7.07
14	*n*-C_4H_9	H	80~87	$C_{58}H_{72}O_8N_6S_4 \cdot 6H_2O$	57.21	6.95	6.90	57.67	6.32	6.37
15	C_2H_5	2-Cl	84~96	$C_{54}H_{62}O_8N_6S_4Cl_2 \cdot H_2O$	56.88	5.66	7.37	56.62	5.60	6.75
16	C_2H_5	2-OCH_3	88~95	$C_{56}H_{68}O_{10}N_6S_4 \cdot 4H_2O$	55.79	6.35	6.97	55.97	6.02	6.77
17	*n*-C_3H_7	3-Cl	80	$C_{56}H_{66}O_8N_6S_4Cl_2 \cdot H_2O$	57.57	5.87	7.19	57.27	6.23	6.98
18	*n*-C_4H_9	3-Cl	70~80	$C_{58}H_{70}O_8N_6S_4Cl_2 \cdot 2H_2O$	57.39	6.10	6.92	57.51	6.55	6.53

8) T. Ito, T. Ishii and M. Nishio, This Journal, **29**, 728 (1965).
9) M. Nishio and T. Ito, *ibid.*, **29**, 732 (1965).
10) M. Nishio and T. Ito, *ibid.*, **29**, 1119 (1965).
11) The preparation of α-ethylthio-(3,4-dimethoxy)-cinnamic acid was reported by Campaigne and Cline. *J. Org. Chem.*, **21**, 32 (1956).

The acids were converted to their chlorides and coupled with 6-APA in aqueous acetone containining sodium bicarbonate. When the acid chlorides were unstable, the mixed anhydride method of coupling[12] was applied.

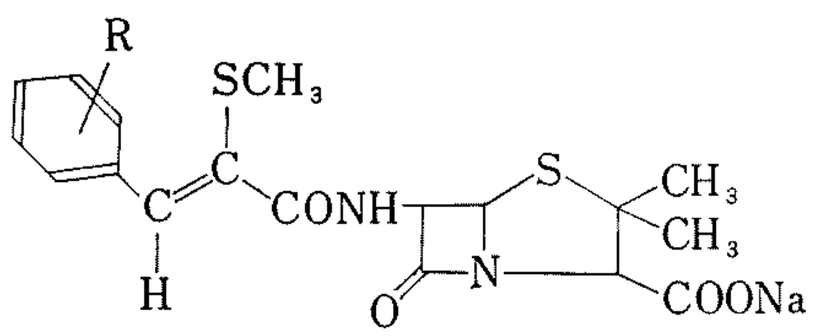
After the reaction, the penicillins were extracted in ether as its free acids and then back into water by adding the requisite amount of sodium hydroxide. Lyophylization of the aqueous phase gave the sodium salts of penicillins, which were sufficiently pure for preliminary biological tests.

N,N′-Dibenzylethylenediamine salts of these penicillins were obtained as needle crystals. The elementary analyses and the decomposition temperatures are listed in Table I and II. The sodium salts were regenerated from the crystalline N, N′-dibenzyl-ethylenediamine salts. The purity of sodium salts of these penicillins was confirmed by the thin layer chromatography.

Antibacterial Properties. Tables III and IV report the minimal inhibitory concentrations of the sodium salts of penicillins for susceptible and resistant strains of staphylococci.

trans-α-Methylthio-cinnamyl penicillin and its analogues (Table III) were highly inhibitory to *Staph. aureus* 209P. The comparison of the

TABLE III. ANTISTAPHYLOCOCCAL ACTIVITY IN VITRO OF *trans*-α-METHYLTHIO-CINNAMYL PENICILLINS (Sodium Salt)

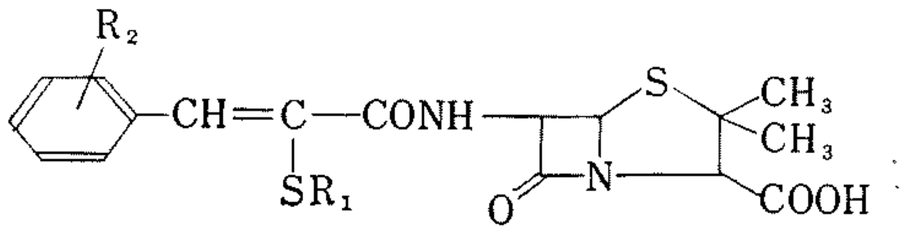


No.	R	Minimum inhibitory concentration (μg/ml)[a]				
		S. aureus 209P	*S. aureus* resistant[b]	*S. aureus* 26[c]	*S. aureus* 101[d]	*S. aureus* BABA[e]
1	H	0.16	5	—	—	—
2	2-Cl[f]	0.08	0.62	25	3.75	—
3	3-Cl	0.39	—	12.5	12.5	
4	4-Cl	1.56	—	6.25	6.25	
5	3, 4-di-Cl	6.25	—	—	—	—
6	2, 6-di-Cl	0.19	1.56	12.5	6.25	0.78
7	2-Br	0.09	1.56	3.12	1.56	0.78
8	4-Br	0.39	12.5	25	12.5	1.56
9	2-OCH_3	0.78	6.25	50	—	1.56
10	2-CH_3	0.19	6.25	12.5	—	—
11	4-CH_3	0.78	—	—	—	—
Benzylpenicillin (K Salt)		0.04	12.5	100	>120	31.3
Phenoxypropyl Penicillin (K Salt)		0.09	—	50	50	0.78
Cloxacillin (Na Salt)		0.19	0.39	0.78	6.25	0.21

a. Two-fold serial dilution technique; m. i. c. read after 48 hr. incubation at 37°C
b. The laboratory-trained benzylpenicillin-resistant strain
c. d. The strains have been isolated from patients in Tokyo Medical and Dental University. The strain 26 was resistant also to dihydrostreptomycin, erythromycin and tetracycline and the strain 101 was resistant also to dihydrostreptomycin and tetracycline.
e. The strain has been isolated from a patient in The Jikei University School of Medicine.
f. See also the references (6) and (7).

12) Y. G. Perron, W. F. Minor, C. T. Haldrege, W. J. Gottstein. J. C. Godfrey, L. B. Crast, R. B. Babel and L. C. Cheney, *J. Am. Chem. Soc.*, **82**, 3934 (1960).

TABLE IV. ANTISTAPHYLOCOCCAL ACTIVITY IN VITRO OF α-ETHYLTHIO-, α-*n*-PROPYLTHIO- AND α-*n*-BUTYLTHIO-CINNAMYL PENICILLINS
(Sodium Salt)



No.	R_1	R_2	Minimum inhibitory concentration (μg/ml) *S. aureus* 209P
12	C_2H_5	H	0.39
13	*n*-C_3H_7	H	1.56
14	*n*-C_4H_9	H	6.25
15	C_2H_5	2-Cl	0.19
16	C_2H_5	2-OCH_3	1.56
17	*n*-C_3H_7	3-Cl	0.39
18	*n*-C_4H_9	3-Cl	0.78

antibacterial activity of α-methylthio-, ethylthio-, *n*-propylthio- and *n*-butylthio-cinnamyl penicillins (**1**, **12**, **13** and **14**) (Table III and IV) showed that when the S-alkyl group became larger, the activity against *Staph. aureus* 209P decreased. Among the substituted α-methylthio-cinnamyl penicillins, ortho substituted penicillins seemed to be more effective than the penicillins which were substituted in para or meta position of benzene ring.

Against resistant strains, *trans*-α-methylthio-2, 6-dichloro-cinnamyl penicillin (**6**) and *trans*-α-methylthio-2-bromo-cinnamyl penicillin (**7**), like *trans*-α-methylthio-2-chloro-cinnamyl penicillin (**2**), were much more effective in vitro than benzylpenicillin.

The inhibitory activity of these penicillins towards resistant strains probably results from the inertness of the compounds to penicillinase.

Stability towards Penicillinase. The in vitro susceptibilities of the selected penicillins to *Bacillus cereus* penicillinase were investigated. The penicillins and penicillinase were dissolved in buffer at pH 7, (kept at 37°C) and the remaining activity of samples was determined by the cylinder method (Table V).

The stability of *trans*-α-methylthio-2-chloro-cinnamyl penicillin (**2**) was reported in the previous paper.[6] The penicillins **4**, **7** and **8**, like the penicillin **2**, were more resistant to *B. cereus* penicillinase than benzylpenicillin. The penicillin **6** was less stable than the penicillin **2** or **7**.

The importance of steric hindrance to the attachment of penicillinase (β-lactamase) has already been discussed by many workers. In α-methylthio-cinnamyl penicillins, the bulky S-methyl group might serve to prevent opening of the lactam ring.

Stability in Acidic Aqueous Solution. The acid stability of several penicillins (sodium salt), including benzylpenicillin and phenoxyethyl penicillin, in citrate buffer at pH 2.0 at 37°C is shown in Table VI. The free acids of α-methylthiocinnamyl penicillins (**2**, **6**, **7** and **8**) were hardly soluble in the buffer at pH 2.0, so they were more stable than benzylpenicillin. The penicillins **2**, **6**, **7** and **8** had essentially the equivalent stability. These compounds were less stable than phenoxyethyl penicillin, however, they were sufficiently stable for oral administration.

The further evaluation of *trans*-α-methylthio-2-bromo-cinnamyl penicillin (**7**), which showed a good in vitro activity, are now under way. The results will be reported elsewhere.

TABLE V. STABILITY OF PENICILLINS TO *B. cereus* PENICILLINASE

Time (minutes)	Residual Penicillin (%)						
	Penicillin **4** Na salt	Penicillin **6** Na salt	Penicillin **7** Na salt	Penicillin **8** Na salt	Penicillin **2** Na salt	Benzyl penicillin K salt	Cloxacillin Na salt
0	100	100	100	100	100	100	100
30	80	55	80	83	84	35	85
60	60	38	63	53	52	0	64

TABLE VI. STABILITY OF PENICILLINS IN THE BUFFER AT pH 2, AT 37°C

Time (minutes)	Residual Penicillin (%)					
	Penicillin **2** Na salt	Penicillin **6** Na salt	Penicillin **7** Na salt	Penicillin **8** Na salt	Benzyl penicillin K salt	Phenoxyethyl penicillin K salt
0	100	100	100	100	100	100
30	70	70	70	72	15	83
60	40	52	50	40	0	60

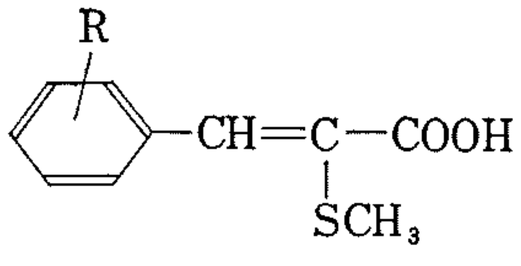
EXPERIMENTAL

The thin-layer chromatograms used in this work were prepared using silica gel "Woelm." The solvent system was benzene, acetone, acetic acid, cyclohexane (55 : 30 : 5 : 10 V), and the zones were spotted with a 0.5% aqueous potassium permanganate solution.[13] The R_F values of sodium salts of α-alkylthio-cinnamyl penicillins were 0.6~0.8.

Substituted α-methylthio-cinnamic acids. The acids listed in the Table VII were prepared newly according to method described in the previous paper,[9] and their geometrical configurations were determined by the NMR spectrum.[9]

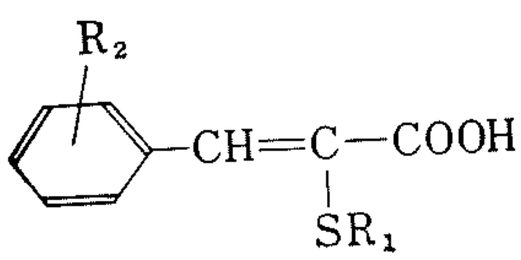
The yield of *cis*-α-methylthio-2-bromo-cinnamic acid

TABLE VII. α-METHYLTHIO-CINNAMIC ACIDS



R	Geometrical configuration	m. p. °C	Formula	*Anal.* Calcd. C	H	S	Found C	H	S
3, 4-di-Cl	*trans*	154~5	$C_{10}H_8O_2SCl_2$	45.64	3.60		45.35	3.40	
2, 6-di-Cl	*trans*	133~5	$C_{10}H_8O_2SCl_2$				46.17	3.30	
2-Br	*trans*	158~9	$C_{10}H_9BrO_2S$	43.97	3.32		43.53	3.51	
	cis	91~2					44.19	3.72	
2-OCH_3	*trans*	123~4	$C_{11}H_{12}O_3S$	58.91	5.39	14.30	58.33	5.82	14.61

TABLE VIII. α-ETHYLTHIO-, α-*n*-PROPYLTHIO- AND α-*n*-BUTYLTHIO-CINNAMIC ACIDS



R_1	R_2	m. p. °C	Formula	*Anal.* Calcd. C	H	S	Found C	H	S
C_2H_5	H	65~6	$C_{11}H_{12}O_2S$	63.43	5.81	15.40	63.16	6.30	15.79
n-C_3H_7	H	66~6	$C_{12}H_{14}O_2S$	64.83	6.35	14.42	64.40	6.38	14.35
n-C_4H_9	H	70~1	$C_{13}H_{16}O_2S$	66.07	6.82	13.57	65.67	6.52	13.92
C_2H_5	2-Cl	71~6	$C_{11}H_{11}ClO_2S$	54.43	4.57		54.60	5.07	
C_2H_5	2-OCH_3	85~1	$C_{12}H_{14}O_3S$	60.48	5.92	13.46	60.57	6.10	13.69
n-C_3H_7	3-Cl	75~7	$C_{12}H_{13}ClO_2S$	56.14	5.10		56.33	5.44	
n-C_4H_9	3-Cl	b. p. 171~3°/1.5 mm	$C_{13}H_{15}ClO_2S$	57.66	5.58		57.60	5.94	

13) D. A. Johnson and C. A. Panetta, *J. Org. Chem.*, **29**, 1826 (1964).

was much lower than that of *trans* isomer, so the properties of *cis*-α-methylthio-2-bromo-cinnamyl penicillin were not yet examined.

α-Ethylthio-, α-*n*-propylthio- and α-*n*-butylthio-cinnamic acids. The acids (Table VIII) were prepared by the following procedure.

To a solution of β-aryl-α-thiopyruvic acid[8,10] (1 mole) in 15% aqueous sodium hydroxide, was added 1.5 mole of ethyl iodide (or *n*-propyl iodide or *n*-butyl iodide) and the solution was refluxed in a boiling water bath for 1 hr. The mixture was poured into ice water containing hydrochloric acid. The separated crystals were filtered, washed with water and recrystallized from a mixture of ethanol and water. When the crude product separated as an oil from ice water, it was extracted with ether and was purified by a distillation under reduced pressure (0.1 ~0.5 mmHg.).

***trans*-α-Methylthio-cinnamoyl chloride.** α-Methylthio-cinnamic acid (3 g) was mixed with thionyl chloride and was refluxed at 55° for 15 minutes. After removal of excess reagent, the residue was distilled under reduced pressure. B. p. 118°C/2 mmHg.; Yield, 2.9 g (88%).

Anal. Calcd. for $C_{10}H_9OSCl$; C, 56.47; H, 4.27, Found: C, 56.21; H, 4.00%.

The following acid chlorides were prepared by the similar procedure. *trans*-α-Methylthio-2-chloro-cinnamoyl, *trans*-α-methylthio-3-chloro-cinnamoyl, *trans*-α-methylthio-4-chloro-cinnamoyl, *trans*-α-methylthio-3, 4-dichloro-cinnamoyl, *trans*-α-methylthio-2, 6-dichloro-cinnamoyl, *trans*-α-methylthio-2-bromo-cinnamoyl, *trans*-α-methylthio-4-bromo-cinnamoyl, α-ethylthio-cinnamoyl and α-ethylthio-2-chloro-cinnamoyl chlorides.

trans-α-Methylthio-2-methoxy-cinnamoyl chloride was used without vacuum distillation.

Acylation of 6-aminopenicillanic acid

Method A. The following procedure illustrates the general method employed for the preparation of the penicillins **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, **9**, **12** and **15**.

***trans*-α-Methylthio-2-chloro-cinnamyl penicillin (2).** To a stirred and cooled solution of 43 g of 6-APA in 1.1 l of water containing 86 g of sodium bicarbonate, was added a solution of 69 g of *trans*-α-methylthio-2-chlorocinnamoyl chloride in 500 ml of dry acetone. After stirring for 1 hr. at 0~5°C, the reaction mixture was extracted with ether. The aqueous phase was separated and covered with 500 ml of ether, cooled in an ice bath, and acidified to pH 2 with phosphoric acid. The ether extract was washed with water and stirred with water while the pH was brought to 6 with 4% sodium hydroxide solution. Lyophylization of the aqueous phase gave the sodium salt of *trans*-α-methylthio-2-chloro-cinnamyl penicillin. Yield, 69 g (73%).

Anal. Calcd. for $C_{18}H_{18}O_4N_2S_2ClNa \cdot H_2O$: C, 46.30; H, 4.32; N, 6.00. Found: C, 46.05; H, 4.29; N, 5.55.

Method B. The penicillins **10**, **11**, **13**, **14**, **16**, **17** and **18** were prepared by the following procedure.

***trans*-α-Methylthio-2-methyl-cinnamyl penicillin (10).** To a stirred and cooled solution of 4.2 g of *trans*-α-methylthio-2-methyl-cinnamic acid in 35 ml of dry terahydrofuran containning 2.8 ml of triethylamine, was added 2.2 g of ethylchloroformate. When the addition had been completed the reaction mixture was stirred for 1 hr. at 0°C and then, with vigorous stirring, a cold solution of 4.3 g of 6-APA and 3 ml of triethylamine in 20 ml of water was added. The reaction mixture was kept stirred for 2 hrs. After a dilution with 20 ml of water the reaction mixture was extracted with methyl isobutyl ketone. The aqueous phase was covered with 50 ml of ether, cooled in an ice bath, acidified to pH 2 with phosphoric acid. The ether extract was washed with water and stirred with water while the pH was brought to 6 with 4% sodium hydroxide solution. Lyophylization of the aqueous phase gave the sodium salt of *trans*-α-methylthio-2-methyl-cinnamyl penicillin. Yield, 3.3 g.

N, N′-Dibenzylethylenediamine salts of α-alkylthio-cinnamyl penicillins. A solution of 3 g of sodium salt of α-alkylthio-cinnamyl penicillin in 15 ml of water was mixed with a solution of 1 g of N,N′-dibenzylethylenediamine diacetate in 15 ml of water. The crystalline precipitates separated at once. After standing in a refrigerator, the precipitates were filtered and washed with water. Recrystallization from acetone-water gave needle crystals. Yield, 80~90%.

Conversion of N, N′-dibenzylethylenediamine salt of α-alkylthio-cinnamyl penicillin to its sodium salt. The suspension of N, N′-dibenzylethylenediamine salt of penicillin in water was mixed with ether, cooled to 0°C, and acidified to pH 2 with phosphoric acid. The mixture was stirred vigorously until all of the solid had dissolved. The ether layer was separated and stirred with water while the pH was brought to 6 with 4% sodium hydroxide. Lyophylization of the aqueous phase gave the sodium salt of penicillin.

Stability towards penicillinase. Five mg of sodium salt (or potassium salt) of penicillin was dissolved in 5 ml of 1/15 M phosphate buffer (pH 7). The solution was mixed with 5 ml of the solution of commercial *B. cereus* penicillinase in the same buffer. The mixture was kept at 37°C. Aliquots of 2 ml was withdrawn at intervals and, after the enzyme was destroyed by placing in boiling water-bath for 1 minute, the remaining activity was determined by the cylinder method.

Stability in acidic aqueous solution. The solution of 4 mg of sodium salt (or potassium salt) of penicillin in 0.4 ml of water was divided in four tubes. In each tube, which contained 0.1 ml of solution, was added 0.9 ml of 1/10 M citrate buffer (pH 2). They were kept in a water bath at 37°C. At intervals, a tube was withdrawn and, after its contents were neutralized with alkali to dissolve the precipitates, the activity was determined by the cylinder method.

Acknowledgement. The authors are grateful to Dr. H. Ogawa for his kind encouragement throughout the work. The authors thank Dr. M. Oda for his interest, Dr. B. Nomiya and his colleagues and the members of Pharmaceutical Division of Kawasaki Factory of this company for the in vitro studies of antibacterial activity. The authors are indebted to Miss T. Ishizuka for experimental assistance.